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Systematic study in *Cressa cretica* L. (Convolvulaceae) species from Lake Qarun, El-Fayoum province, Egypt

Wafaa Kamal Taia [1] and Amany Mohamed Abd El-Maged [2,*]

[1] *Department of Botany and Microbiology, Faculty of Science, Alexandria University, Egypt.*
[2] *Department of Biology and Geology, Faculty of Education, Alexandria University, Egypt.*

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Abstract

Cressa cretica L. is a monotypic species belongs to the plant family Convolvulaceae, tribe Cresseae. It is an erect, small, dwarf shrub, commonly grown in coastal areas and considered as an important medicinal plant with many therapeutic effects. Individuals of this species became restricted in specific areas in Egypt in became under threat of extinction. This study dealt with careful examination of the external and leaf internal structure beside pollen grain morphology. The study aims to understand the species morphological characteristics and protect it from localization and destruction processes. The results showed that the leaf macro- and micro-morphological characters are of the halophytic ones, with long unicellular hairs as well as T-shaped hairs and sunken stomata. The leaf internal structure has mesophyll differentiated into one layer palisade and spongy layers full of ca-oxalate crystals and salt glands distributed in the mesophyll. Pollen grains productivity was few and they are small, spheroidal to subprolate with tricolporate aperture and tectate perforate with supra-tectum granules. The results obtained show that *Cressa cretica* has external and internal halophytic structure in addition to few small pollen grains. Germination experiments needed to test the pollen productivity of this plant under non-saline habitats for its conservation.

Keywords: *Cressa cretica*; Leaf anatomy; Leaf morphology; Pollen grains

1 Introduction

Cressa cretica L. is a perennial stout halophytic subshrub grown to a height of about 40 cm above ground surface with very long underground stem (rhizome) reaching more than a meter [1]. It belongs to family convolvulaceae with wide distribution in the coastal salt-marches worldwide [2]. Members of this family distinguished by their five-lobed funnel-shaped petals and by their reproductive structures. Individuals of this species usually grow in sandy or muddy habitats with high salt contents in association with *Suaeda maritima*, *Salicornia europaea*, *Salsola soda*, *Limonium vulgare* subsp. *Serotinum*, and *Crypsis aculeate* [3]. This species has been taxonomically handled in three ways, 1- considered as monotypic species [4, 5, 6, 7], 2- treated as 19 species by splitting every morphological variant into single species [8, 9, 10, 11, 12], 3- treated by [13] as four species, two endemic to America (*C. truxillensis* and *C. nudicaulis*), one in Eurasia and Africa (*C. cretica*), and one (*C. australis*) in Australia. Accordingly individuals of *C. cretica* modulate with their habitats, but generally they have its specific morphological features that made it adapt with both salinity and dryness [14]. It grows during the dry phase of transient marches, as it develops over the hot and dry summer and stop when water return. Individuals of this species have compact, small, sessile woolly leaves and clustered, sessile white or creamy flowers grouped in short spikes from the axils of the upper leaves [4, 7, 15]. *Cressa cretica* considered as an important medicinal plants with many therapeutic effects [16, 3], listed in the IUCN Red list as threatened species [17, 18].

* Corresponding author: Amany Mohamed Abd El-Maged

In Egypt, this species was very common found in different habitats from the Mediterranean coastal region. It was found widely in all the sandy and salty habitats from Port Said to El-Saloum beside the Nile Delta, Oasis, deserts, and Gebel Elba. Unfortunately, individuals of this species become restricted in few areas in Egypt [4, 6]. Individuals of this species can be found in sparse patches in hot dry areas near the dry lakes and sandy salt marches. In an attempt to understand the external and internal characters of this species, the plant gathered near Lake Qarun, El-Fayoum province, Egypt examined carefully externally and internally to understand its morphological characteristics and protect it from localization and destruction processes.

This work has been done to investigate the main external and internal characters of *Cressa cretica* to assess its taxonomic relation with the other convolvulus taxa. In the same time the complete knowledge of this important medicinal plant and its morphological adaptation to aridity and salinity will enable its protection and conservation.

2 Material and methods

2.1 Plant specimens

Field trips carried from May to June 2023 near Lake Qarun area in El-Fayoum province to recognize the plant communities in association with *Cressa cretica*. Plant specimens collected from the dry sandy habitat near the lake (Figures 1 & 2).

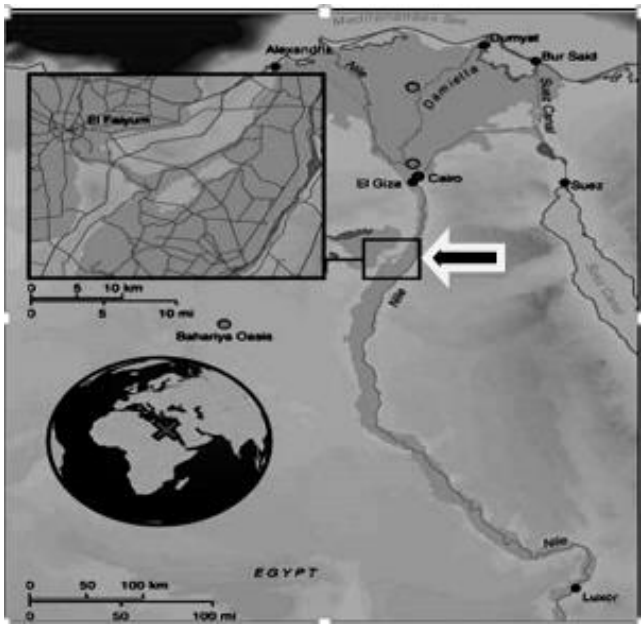


Figure 1 El-Fayoum province, Egypt

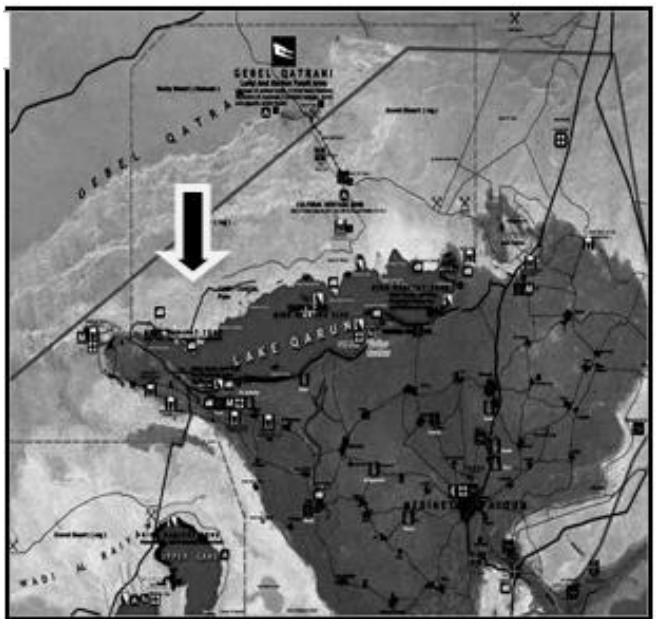


Figure 2 Lake Qarun in El-Fayoum

2.2 Macro-morphological study

The macro characters assessed on mature leaves; from five fresh plant individuals; from the middle of the stem. Leaf length and width measurements were carried by ruler. Pieces of matured leaves were put onto cleaned Aluminum stubs, coated with gold and examined under the Scanning Electron Microscope. Leaf apex, margin, shape, surface, venation and base described according to [19].

2.3 Micro-morphological study

For leaf micro-morphological investigation, perfect leaves were chosen for prior examination under stereomicroscope to estimate the hair density and leaf texture. Then, the middle part from both the abaxial and adaxial surfaces was heated gently in water with a few drops of both Tepol and nitric acids to peel the epidermal layers for epidermal cells, stomata, and hair type. The stomatal density calculated by the number of stomata/ area (Density of stomata mm2) = SN/A, where SN = stomata number; A = area (mm2). Terminology used in the stomata description is that of [20]. The examination and photographs done by using Motic (B-150D) light microscope fitted with USB digital-Video Camera and Computer Software with 10X40 lenses. Leaf micro-morphological descriptions followed [21, 22].

2.4 Leaf anatomy

Transverse sections in the fifth leaf from the terminal bud was carried followed the procedure of [23]. The leaf was dehydrated in series of alcohol, stained with toluidine blue, ended with three changes of xylene with alcohol 1:1, then xylene with paraffin wax 1:1 and finally the leaf was embedded in block of pure paraffin and sectioned by the aid of Rotary Microtome. The thickness of the sections was 10–15 µm. The sections photographed by the same light microscope.

2.5 Pollen morphology

Mature unopened flowers were carefully opened using forceps and needles to get the anthers, non-acetolysed pollen grains were sputtered onto glass slide in few drops of glycerol media, to investigate the morphological characters of the pollen grains. Polar, Equatorial, Aperture type and number beside all the measurements were done using Ocleometer fitted in 10X lens and photographed using Motic (B-150D) light microscope fitted with USB digital-Video Camera and Computer Software with 10X40 lenses. The measurements recorded in 30 pollen grains to calculate the average and standard deviation. For Scanning electron microscope investigation, non-acetolysed pollen grains have been sputtered onto cleaned, Aluminum labeled stubs furnished by double sticky cello tape then coated with 20 nm Gold in a Polaron JFC-1100 coating unit, examined and photographed using JEOL-JSM.I T200 Series SEM allocated in the electron microscope unit, Faculty of Science, Alexandria University, Egypt. The terminology used here is that of [24].

3 Results

Cressa cretica individuals are found as small shrubs as sparse populations in salt march vegetation near Lake Qarun (Figures 3-7). Other halophytic species as *Alhagi maurorum, Tamarix nilotica* and *Nitraria retusa* are found in close communities beside it. The leaf macro-morphological characters and internal structure beside pollen morphological characters are listed in table 1.

3.1 Leaf macro-morphology

The leaves are stipulate; with two filamentous small stipules adjacent to the stem; sessile, alternate. The leaf blade simple, pale green to grayish, small where their lengths varied from 0.2-0.6 cm and width from 0.1-0.3 cm (Figures 3, 4, 5, 6, 8, 9). They are lanceolate to broadly lanceolate, with scariosus texture i.e. thin dry shriveled appearance, covered with long hairs in both surfaces (Figures 4, 5, 7). Leaf blade base is truncate with entire margin and acute apex (Figures 4, 5). The venation is eureticulodromous where there is one main midrib and ramified lateral veins reaching to the margins.

3.2 Leaf micro-morphology

The epidermal cells are isodiametric in both the adaxial and abaxial surfaces (Figures 11, 13). The leaf blades in both adaxial and abaxial surfaces covered with long unicellular (Figures 8, 9, 10, 12), uniseriate pointed hairs and bi-forked T-shaped hairs (Figures 8, 9) which are distributed all over the adaxil surface and restricted around the midrib and lateral veins in the abaxial surface. The leaves are amphistomatous i.e. the stomata are in both adaxial and abaxial surfaces. Stomata are mainly of the anisocytic type in both surfaces, but paracytic stomata have been found in the adaxial surface (Figures 11, 13). The guard cells are elliptical with elongated pores (Figures 11, 13). The calculated stomatal density was 121.8 in the adaxial surface and 138.2 in the abaxial one. Wide cavities were noticed in both the adaxial and abaxial surfaces.

3.3 Leaf anatomy

The leaf blade is thin with undulate surface, and without elevated mid rib. The epidermal cells in both two surfaces; which consist of one layer; have rectangular or rounded shape cells and become cubical at both ends (Figures 17, 19). The epidermal cells have different sizes and their wall thickness varied between both the adaxial and abaxial surfaces (Figures 16-19). The epidermis has sunken stomata beside the superficial ones (Figures 14, 18, 19). The mesophyll is distinguished into palisade and spongy tissue, the palisade tissue consists of one layer of elongated columnar cells filled with plenty of chloroplasts (Figure 14). While the spongy tissue consists of 2 to 3 irregular interspaced parenchyma cell layers contain few chloroplasts (Figure 14). The two mesophyll tissues have enlarged schizogenous cavities as well as druses of calcium oxalate (Figures 20, 21). The two ends of the leaf blade in T.S. are circular with angular or lacunate collenchyma cells underneath. The mid rib and lateral veins regions are in the same level of the leaf blade (Figures 14, 15). The vascular bundles are either of the collateral or bicollateral types, consist of few thin xylem vessels and small patch of phloem abaxially and sometimes few phloem cells found adaxially (Figure 17).

3.4 Pollen morphology

The pollen grains are very few in all the flower stages. Pollen grains are isodiametric, radially symmetric, spheroial or subprolate, small with polar axis length from 22.8-30.4 µm and equatorial axis length from 20.8-26.2 µm, prolate pollen grains have been recorded (Figures 27, 29). Aperture tricolporate, with short narrow colpi (Figures 30, 32), and smooth edges (Figures 22-26). Exine considerably thin (0.6-0.8µm) with tectate perforate ornamentation with supra-tectum granules (Figure 31). Some pollen grains have tectate exine with dense granules (Figure 28).

Table 1 Characters investigated within *Cressa cretica* species

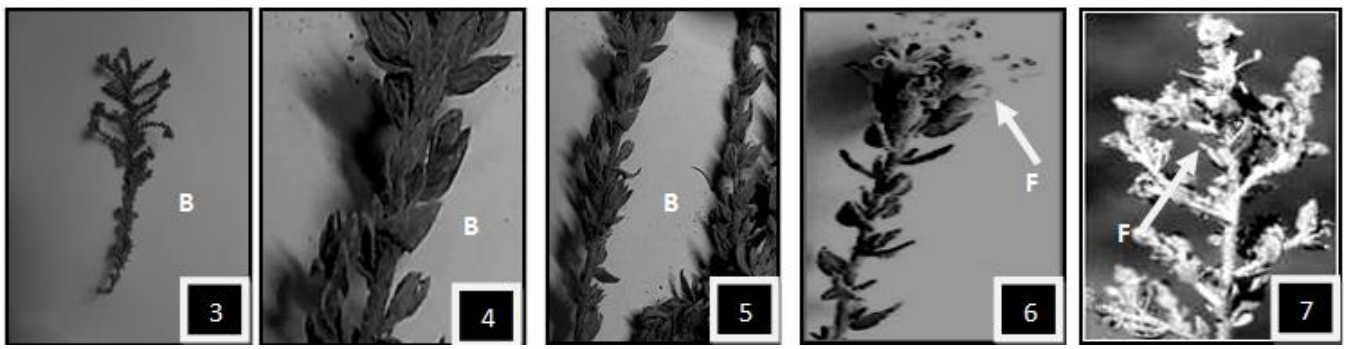
Leaf macro-morphology (measurements in cm)												
Stipules	**Petiole**	**Arr**	**Type**	**Blade length (L)**	**Blade width (W)**	**L/W**	**Shape**	**Texture**	**Surface**	**Ven**	**Margin**	**Apex**
Two Filamentous	Sessile	Alternate	Simple	0.2–0.6 (0.4±0.2)	0.1–0.3 (0.2±0.1)	1.75	Lanceolate. Broadly lanceolate	Scarious	Woolly	ER	Entire	Acute

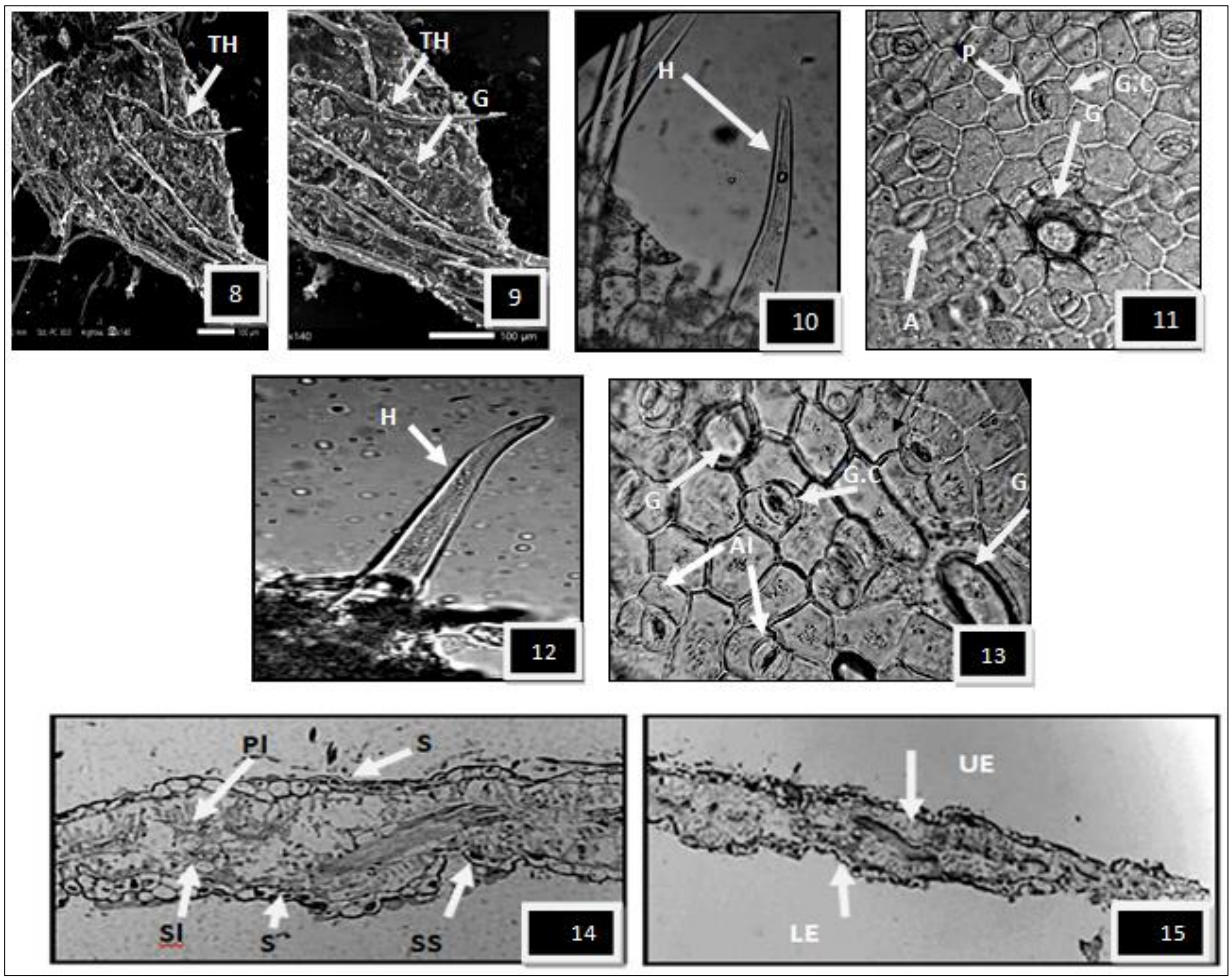
Leaf micro-morphology (light microscope)											
Adaxial surface						**Abaxial surface**					
Epidermal cells		**Hairs**		**Stomata**		**Epidermal cells**		**Hairs**		**Stomata**	
Shape	**AW**	**Pos**	**Type**	**Type**	**Density**	**Shape**	**AW**	**Pos**	**Type**	**Type**	**Density**
Iso	Straight	All	UP	Paracytic anisocytic	121.8	Iso	Slightly wavy	M&V	UP	Anisocytic	138.2

Pollen grain morphology									
Polarity	**Symmetry**	**Polar axis (PA)**	**Equatorial axis (EA)**	**PA/EA**	**Shape**	**Aperture type**	**Aperture number**	**Exine thickness**	**Exine orn**
Iso	Symmetric	22.8-30.4 (28.7±1.7)	20.8-26.2 (24.8±1.4)	1-1.13	Spheroidal/subprolate	Colpate	3	0.6-0.8± 0.68	TP/ Granulate

Abbreviations: All=Allover, Arr=Arrengement, AW=Anticlinal wall, ER=Eureticulodromous, Iso=Isodiametric, M=Midrib, orn= ornamentation, Pos=Position, TP=Tectate perforate, UP=Uniseriate pointed, V= Veins, Ven=Venation.

3.5 Photographs of *Cressa cretica* showing morphological, leaf micro-morphological, leaf anatomical and pollen morphological characters

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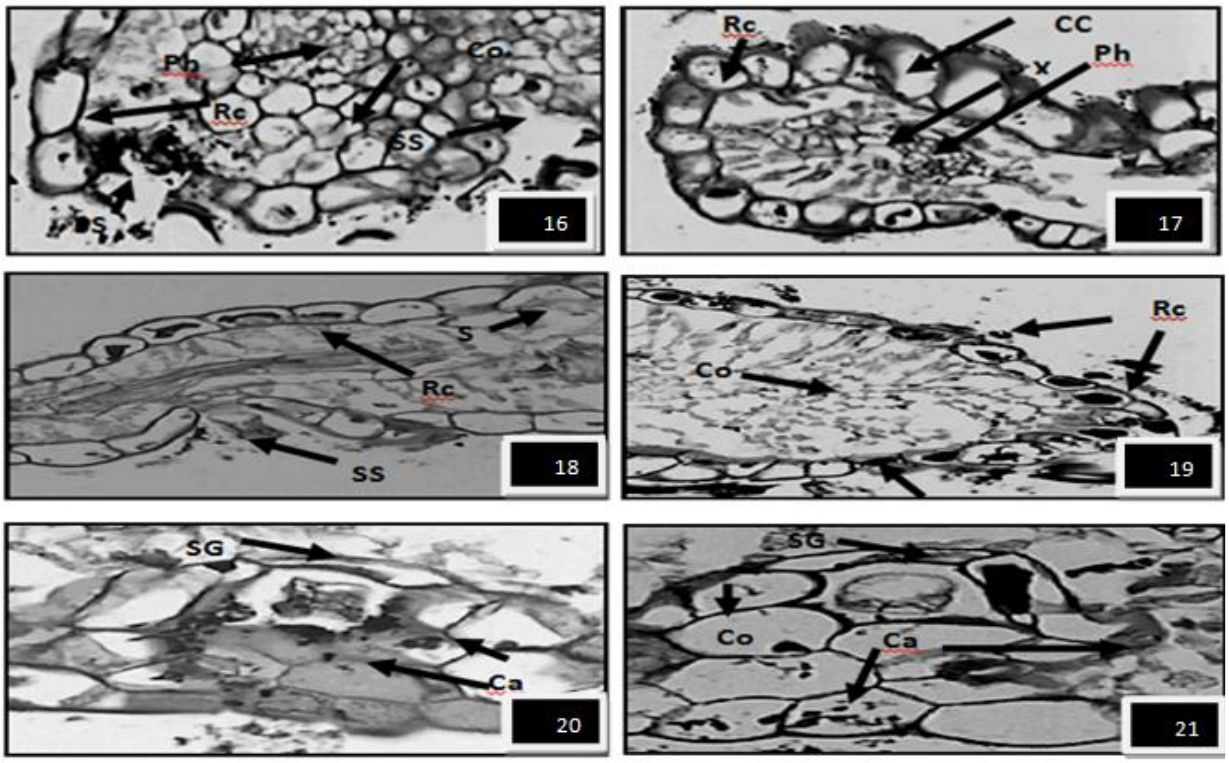
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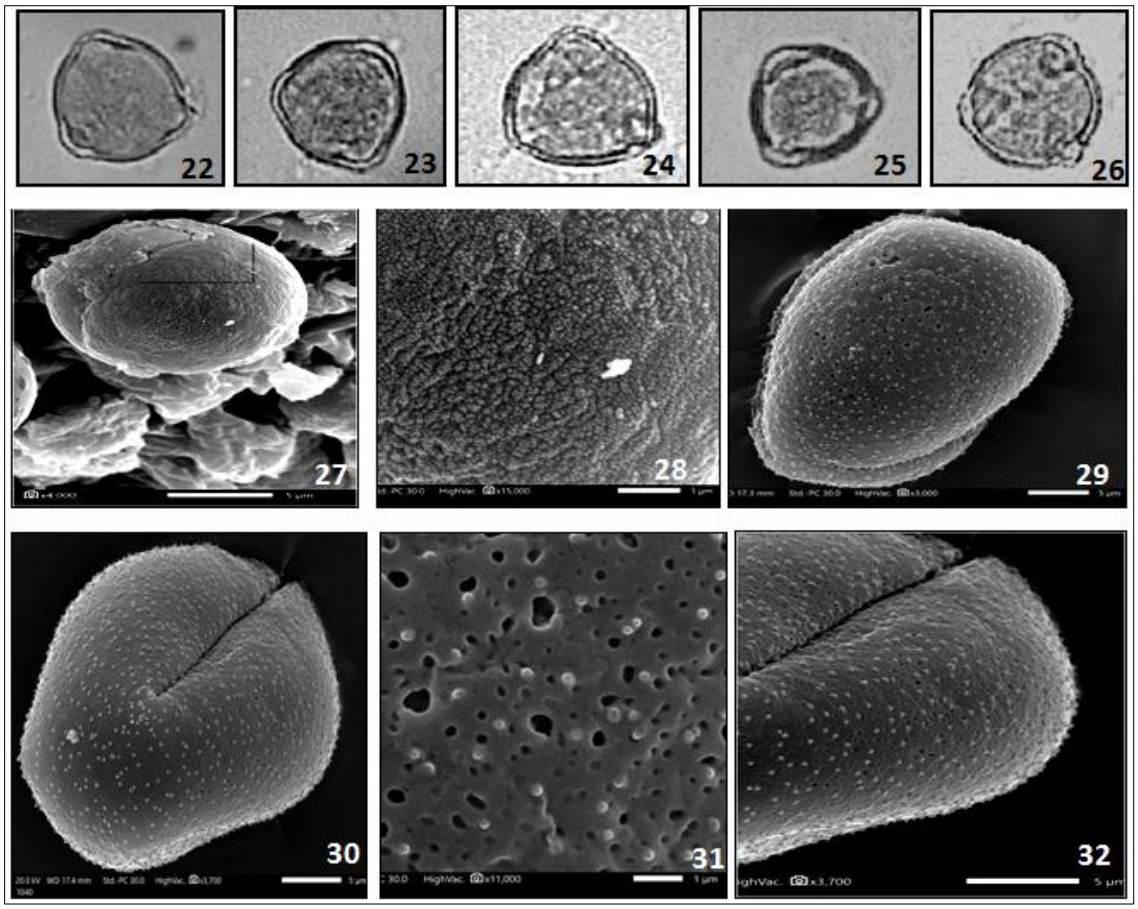

Abbreviations: A=Anomocytic, AI=Anisocytic, B=Branch, Ca=Ca-oxalate, CC=Cubical cell, Co=Collenchyma cells, F=Flower, G=Gland, Gc=Guard cell, H=Hair, LE=Lower epidermis, P=Paracytic, Ph=Phloem, Rc=Rectangular cells, S=Superficial stomata, SG=Schizoginous gland, Sl=Spongy layer, SS=Sunken stomata, TH=T-shaped hair, UE=Upper epidermis, X=Xylem. LM photographs for hairs, stomata, anatomy and pollen grains are under 40X magnification except photo 14 (10X) and 15 (5X).

Figures (3-7) External features of *cressa cretica* species. Figures (8, 9) SEM Photographs of leaf surface. Figures (10-13) Leaf epidermal surface under light microscope showing hairs and stomata. Figures (14- 21) Internal structure of leaf under light microscope. Figures (22-26) Light micrographs of pollen grains showing tricolporate aperture. Figures (27-32) SEM Photographs of pollen grains showing colpi and exine ornamentation

4 Discussion

Cressa cretica is a thermo-cosmopolitan halophilous species found along the Mediterranean coastal regions in sandy and saline habitats. It was very common species in Egypt as mentioned by [4]. As the result of urbanization, human influences, tourism and draining of wetlands this species listed by [18] as endangered species and its presence becomes in few areas in Egypt as Lake Qarun. Genus *Cressa* represented by one species in Egypt (*Cressa cretica*) belongs to family convolvulaceae tribe Cresseae [25]. The genus has its characteristic morphology and habitat requisites which made its position with under the convolvulaceae depend mainly on floral characters, especially number of stigma and ovules basal placentation [26].

Most of the research works on this genus are related to the medicinal uses of its species and few were related to its taxonomical status. Accordingly the cited references were considerably old in this concern. The results obtained showed that the macro-morphological leaf characters are suitable for the salty habitats. The leaves are sessile, alternate, and simple with lanceolate leaf blades with scariosus texture and covered with long unicellular pointed hairs. These leaf characters coordinate with the other seven genera under tribe Cresseae; *Bonamia, Cladostigma, Evolvulus, Hildebrandtia, Seddera, Stilisma* and *Wilsonia*; which differs from the other eleven tribes by their leaf and stigma characters [26]. The leaf micro-characters; as seen under the light microscope; revealed that the leaves have isodiametric epidermal cells with straight or slightly wavy anticlinal walls, covered with both long unicellular pointed hairs and bi-forked T-shaped hairs on the adaxial surface and beside the veins on the abaxial surface. These results are in partial agreement with [27] who recorded few hairs in the midrib region of the leaf. The leaf blades are

amphistomatous, with either sunken or superficial stomata on both surfaces. The stomata are of the anisocytic type in the abaxial surface, while paracytic stomata recorded beside the anisocytic and anomocytic ones in the abaxial surface. These stomatal types have been recorded before by [28] but they did not record the paracytic ones. The data obtained are in partial agreement with [29], but they recorded the paracytic stomata as the most abundant type than the anomocytic ones. The stomatal density is slightly differs between the adaxial and abaxial surfaces which is mentioned before by [27]. The occurrence of different types of stomata on the same surface of the leaf in this family is also noted by [29].

Halophytes are plants with particular morpho-anatomical structures that allow them to survive under the stress of salt concentration. These structures are either externally, like reducing leaves and presence of hairs on both leaves and stem to eliminate evapotranspiration, or internally like the presence of salt glands [30]. The internal structure of the halophytic plants was mainly on stem anatomy due to either lake of leaves or succulent small ones. *Cressa cretica* has sessile small simple leaves; accordingly its internal structure has been investigated in this work. The results obtained showed one-layer cubical or spherical shaped epidermal cells with both sunken and superficial stomata on both surfaces. The two stomatal elevations can be related to physiological aspects of the plants. The midrib and all the veins are at the same level of the lamina and this is coordinate with that found by [31].

The palynological investigation on *Cressa cretica* is very few and this is may be due to the low pollen production of halophytes as mentioned by [32]. In this concern [33] found that the pollen production of the same species was few in the saline habitats than that in non-saline ones. The flowers are perfect, and the anther has few pollen grains and this was noticed by [34], as the different developmental anther stages give different pollen grains. In spite of that, under the light microscope the general appearance of them was small, prolate spheroidal to sub prolate. This pollen character is due to the saline habitats.

5 Conclusion

This study proved that *Cressa cretica* species has typical halophytic characters which support its individual to live in saline habitats. The low pollen grain productivity may affect its reproduction and sustainability. Germination experiments needed to test the pollen productivity of this plant under non-saline habitats for its conservation.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

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Author's short Biography

	**Prof. Dr. Wafaa Kamal Taia**, Faculty of Science Alexandria University. taia55taxonomy@hotmail.com, She is graduated from faculty of Science-Alexandria University-Botany Department. Her M.Sc. Was on Palynological study and plant taxonomy, under the supervision of Prof. Dr. Shokry Ibrahim Saad. She got her Ph.D. from Reading University, U.K. in Plant taxonomy under the supervision of Prof. V.H. Heywood.
	**Dr. Amany Mohamed Abd El-Maged**, Faculty of Education Alexandria University. monytag86@gmail.com. She is a lecturer in Biology and Geology Department, Faculty of Education, Alexandria University She received a BSc degree in Biology from the same university. She received MSc and PhD degrees in Plant taxonomy and palynology from Faculty of Science, Alexandria University.